Journal of Chromatography, 461 (1989) 149–163
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1472

MEASUREMENT OF STATISTICAL MOMENTS OF RESOLVED AND OVERLAPPING CHROMATOGRAPHIC PEAKS*

MARK S. JEANSONNE and JOE P. FOLEY*
Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803-1804 (U.S.A.)

SUMMARY

A method for the accurate calculation of statistical moments, excess and skew is described. Based on peak width, asymmetry and peak height measurements this method is applicable to both fully resolved and overlapping chromatographic peaks. It is also useful for the deconvolution of overlapping chromatographic peaks. The advantages of this method over the traditional approach to measuring peak statistical moments, excess and skew are discussed.

INTRODUCTION

The importance of statistical moment analysis to the chromatographer cannot be overemphasized because a large amount of information can be derived from such an analysis. Statistical moment analysis cannot only be used to measure directly parameters such as area (zeroth moment), peak centroid (first statistical moment) and variance (second statistical moment), but other important parameters can be calculated indirectly as well. For example, column efficiency can be calculated from $N = M_1^2/M_2$, where N is the column efficiency, M_1 is the first statistical moment and M_2 is the variance. Other parameters, such as the third and fourth statistical moments give information on peak asymmetry and peak flattening, respectively. Peak skew and excess are parameters related to statistical moments and provide a measure of the deviation of the chromatographic peak from a Gaussian peak profile.

Traditionally, statistical moments for digitally represented chromatographic peaks have been approximated by the simple summation of the magnitude of the peak signal at each data point between the peak start and stop limits, as shown in Fig. 1a. However, any approach based on summation for the calculation of statistical moments has several shortcomings when applied to real chromatographic data.

First, it has been shown that the accuracy and precision of the summation method is directly affected by the amount of noise present in the chromatogram[1,2]. The noise level has been shown to affect peak start/stop assignments, and this affects the limits of summation and, consequently, the value of the statistical moments calculated[3].

* Presented in part at the *Pittsburgh Conference and Exposition in New Orleans, LA, February 1988*, paper No. 088.

0021-9673/89/$03.50

(a)

$$M_0 = \text{area} = \int_{-\infty}^{\infty} h(t)\,dt \approx \sum_{\text{start}}^{\text{stop}} h(t)\,\Delta t$$

$$M_1 = \text{retention time} = \int_{-\infty}^{\infty} t \cdot h(t)\,dt \approx \sum_{\text{start}}^{\text{stop}} t \cdot h(t)\,\Delta t$$

$$M_2 = \text{variance} = \int_{-\infty}^{\infty} (t - M_1)^2 \cdot h(t)\,dt \approx \sum_{\text{start}}^{\text{stop}} (t - M_1)^2 \cdot h(t)\,\Delta t$$

$$M_3 = \int_{-\infty}^{\infty} (t - M_1)^3 \cdot h(t)\,dt \approx \sum_{\text{start}}^{\text{stop}} (t - M_1)^3 \cdot h(t)\,\Delta t$$

$$M_4 = \int_{-\infty}^{\infty} (t - M_1)^4 \cdot h(t)\,dt \approx \sum_{\text{start}}^{\text{stop}} (t - M_1)^4 \cdot h(t)\,\Delta t$$

$$\gamma_S = \text{skew} = M_3/{M_2}^{3/2}$$
$$\gamma_E = \text{excess} = M_4/{M_2}^2 - 3$$

(b)

$$\sigma_G = \frac{W}{f_1(b/a)}$$

$$M_2 = W^2 \cdot f_2(b/a)$$
$$M_1 = t_r + W \cdot f_3(b/a)$$

$$\tau = \sqrt{M_2 - \sigma_G^2}$$

$$M_3 = 2\,\tau^3$$
$$M_4 = 3\,\sigma_G^4 + 6\,\sigma_G{}^2\tau^2 + 9\,\tau^4$$
$$\gamma_S = M_3/M_2^{3/2}$$
$$\gamma_E = M_4/M_2^2 - 3$$

Fig. 1. Equations used for calculation of statistical moments and other peak parameters by (a) the summation method and by (b) the width-asymmetry method. t_r = retention time. $h(t)$ = chromatographic peak profile.

Secondly, the accuracy of the summation method (equations shown in Fig. 1a) deteriorates rapidly as the peaks begin to overlap. We have recently shown[4] that errors in peak area can exceed 100% when the summation approach (perpendicular drop algorithm) is applied to overlapping peaks. As we shall show later in this report, errors in the higher moments calculated via the summation method are usually much larger under the same circumstances.

A final drawback of the summation method is that it is computationally intensive, requiring numerous calculations (see Fig. 1a) for every data point in the peak

of interest. This is particularly true for the higher moments and related parameters. Although this problem has been alleviated somewhat by the advances in computer technology (faster computations), the summation method remains noticeably time-consuming on many commercial chromatographs with microcomputer-based data systems.

Most, if not all, of the problems associated with the measurement of statistical moments can be reduced or eliminated if one has an accurate model for the chromatographic peaks of interest. A model that has been reported to be accurate for most chromatographic peaks[5–7] is the Exponentially Modified Gaussian (EMG) function, which is the convolution of a Gaussian and an exponential decay function. Recently, we introduced[8] a convenient procedure for determining whether or not the use of the EMG model is appropriate. This procedure utilizes empirical equations for calculating peak area based on peak width, asymmetry and peak height measurements. Once the validity of the EMG model for a given set of peaks has been confirmed, these equations can also be used for the accurate measurement of peak areas of overlapping chromatographic peaks[4]. Note that this method relies on the measurement of peak width and asymmetry for the less distorted peak of the overlapped pair (the first peak) at a point above the valley where distortion from the second peak is low.

Although some of the problems associated with the traditional measurement of statistical moments can be reduced or eliminated via the use of a variety of sophisticated, curve-fitting/deconvolution procedures, these procedures also have numerous drawbacks. First, they are nearly always even more time-consuming than the traditional summation approach. In many cases a final summation step is required after the preliminary curve-fitting/deconvolution procedures. Secondly, some of the procedures require multi-channel detection which is not always available. Thirdly and most importantly, for a variety of reasons the curve-fitting/deconvolution approaches have not yet proven to be sufficiently reliable. For example, with iterative procedures, lack of convergence is frequently observed. In general, these and other disadvantages have dissuaded most, if not all, commercial manufacturers from implementing the curve-fitting/deconvolution approaches into their chromatographic data systems.

The purpose of this paper is to report an alternative to both the traditional and least squares/deconvolution methods for the measurement of statistical moments. Our present approach utilizes empirical equations (Fig.1b) similar to those we already reported for peak area[7], but also includes a very simple deconvolution procedure for a pair of overlapping peaks. The derivation of these equations will not be included here, as this topic will constitute a separate paper[9]. For the remainder of this report, we will refer to our method of statistical moment measurement as the width-asymmetry method.

EXPERIMENTAL

Both an Apple Macintosh Plus and an IBM PC-AT were utilized for simulated peak generation and other calculations. All programs were written in either Microsoft BASIC or TRUE BASIC.

EMG peak generation

All peaks generated were based on the EMG function[5] expressed as

$$\mathrm{EMG}(t) = \frac{A}{\tau} \cdot \exp\left[\frac{1}{2}\left(\frac{\sigma_G}{\tau}\right)^2 - \frac{t - t_G}{\tau}\right] \int_{-\infty}^{z} \frac{\exp\left(\frac{-y^2}{2}\right)}{\sqrt{2\pi}} \mathrm{d}y \qquad (1)$$

where A is the peak area, t_G is the retention time, σ_G is the standard deviation of the Gaussian function, τ is the time constant of the exponential decay function convoluted with the Gaussian function and $Z = (t - t_G)/\sigma_G - \sigma_G/\tau$. As the τ/σ_G ratio increases, the peak in question will become more skewed, and as it decreases, the peak approaches a Gaussian shape.

Single chromatographic peaks at τ/σ_G ratios of 0, 0.5, 1, 2, 3 and 4, with σ_G a constant at 0.1 min, were generated for this study, using a sampling rate of three points per second. As shown in Fig. 2 ($\tau/\sigma_G = 2$), about 30 points per peak measured from 10% peak height to 10% peak height were needed for $\leqslant 2\%$ error.

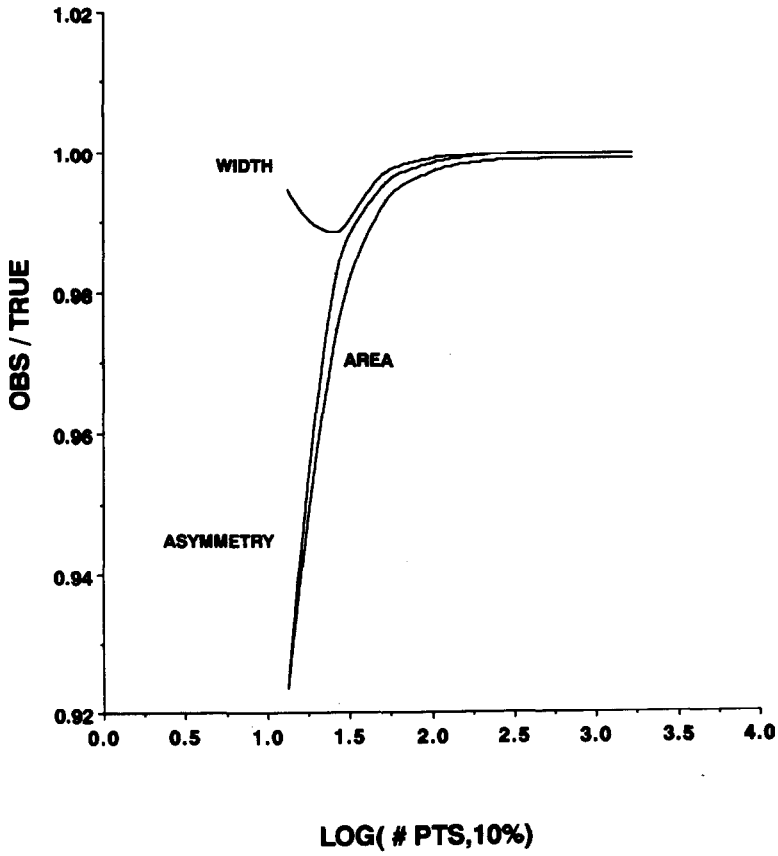

Fig. 2. Effect of the data sampling rate on the measurement of peak width, asymmetry and peak area.

Overlapped chromatographic peaks at τ/σ_G ratios of 0.5, 1, 2, 3 and 4 were generated at resolution values of 0.625, 0.75, 0.875, 1, 1.125, 1.25, 1.375 and 1.5 by using the same sampling rate as the single peaks. Resolution was defined as $\Delta t_G/4(\text{variance})^{1/2}$, where $\Delta t_G = t_{G,2} - t_{G,1}$, and variance was defined as $\sigma_G^2 + \tau^2$ for an exponentially modified Gaussian peak. The peaks were overlapped by adding two individual, simulated peaks of equal area and τ/σ_G value. However, the degree of peak overlap will be reported here as the percent valley due to the inadequacy of the resolution parameter for fully describing tailed overlapped peaks[4]. Percent valley was defined as $h_v/h_p \cdot 100\%$, where h_v and h_p are shown in Fig. 3.

Real chromatographic peak generation

Real single and overlapped pairs of peaks were generated on a Series 400 liquid chromatograph (Perkin-Elmer, Norwalk, CT, U.S.A.), using pyrene as the analyte. The mobile phase composition was 75% aqueous acetonitrile at a flow-rate of 1.5 ml/min. The column used was a Vydac pH-stable C_8 column. A Model V[4] variable wavelength ultraviolet absorption detector (Isco, Lincoln, NB, U.S.A.), set at 330 nm was used to detect pyrene. An Omega-2 data system (Perkin-Elmer) utilizing an IBM AT computer was used for storage of the chromatograms.

Overlapped peaks were obtained from precise, rapid duplicate injections of a standard solution of pyrene. This single-standard, rapid, duplicate-injection approach has many advantages over a two-component standard solution method which would require changing conditions to obtain different degrees of overlap. First, it allows the degree of peak overlap to be easily controlled by simply varying the length of time between injections. Secondly, this method avoids any relative change in the molar absorptivities of two analytes in the mixture as mobile-phase conditions are changed to obtain different degrees of overlap. Thirdly, it permits a single peak to be obtained under the same conditions as the overlapped peaks, thus allowing the statistical moments measured by the summation and width-asymmetry methods for the isolated peak to be compared with those measured for the overlapped peak pair without any concern about changes in the peak shape and/or concentration. Finally,

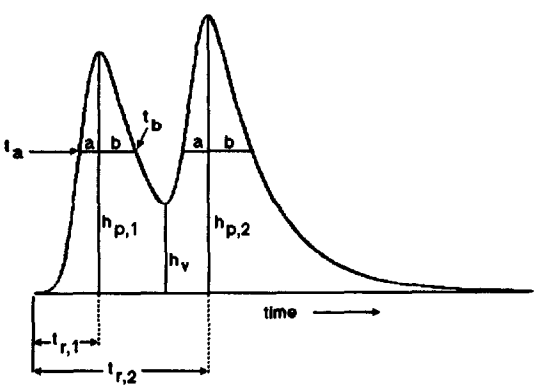

Fig. 3. Measurement of graphic parameters for an overlapping pair of chromatographic peaks. t_r and h_p are the retention time and peak height of respective peaks, and h_v is the height of the valley. Peak width at the desired peak height fraction is given by $t_a - t_b$.

one can be confident that the true area ratio of the overlapped peaks is unity, since equal amounts of the same compound are being injected.

Two pairs of tailed, overlapping peaks with percent valleys of 40 and 67% were generated. A single control peak with the same amount of peak tailing as the overlapping peaks was also generated. The amount of peak tailing was adjusted by adding or removing dead-volume ahead of the column.

Peak parameter measurement

The equations given in Fig. 1a, for determining the zeroth through fourth statistical moments by summation, were applied to both simulated and real peaks. For the simulated peaks, start/stop assignments (limits of integration) for isolated peaks were taken as the point where the peak was determined to be "on baseline", which depended on the "baseline level" being used for the peak. Baseline levels of $1 \cdot 10^{-9}$ and $3 \cdot 10^{-3}$ were used, which corresponded to approximately 0.00 and 0.1% of the peak height, respectively. The lower baseline was used as an ideal baseline in order to obtain a maximum level of accuracy for purposes of comparison. The ideal baseline was chosen to be slightly above zero, since the value of Z in eqn. 1 necessary to give a zero baseline would result in an overflow condition. This ideal baseline was used only for peaks in which no noise was present, since noise affects peak/start assignments in real chromatograms.

For overlapped peak pairs, the starting point for the first peak and stopping point for the second peak were chosen as for isolated peaks. The peak stop for the first peak and the peak start for the second peak were taken as the intersection of the baseline being used and a perpendicular line, drawn to the minimum of the valley between the peaks (see Fig. 3). This method for dealing with overlapped peaks is commonly referred to as the perpendicular drop algorithm.

For the real chromatographic peaks, the baseline level and the start/stop assignments were determined by the data system, the perpendicular drop method being employed for overlapped peaks. The peak detection algorithms in the data system were optimized for the types of real peaks that were generated.

The widths of the peaks at 10, 25, 50 and 75% relative peak height were determined by utilizing a four point least squares fit where four points on each side of the peak, symmetric about the particular height, was used and the difference in time between the two points ($t_a - t_b$) was taken as the peak width (see Fig. 3). Four points were used, since the accuracy of the value obtained for peak width did not increase when more points were fit.

Peak height was obtained by subtracting the baseline value being used from the peak maximum obtained via a quadratic least-squares curve fit of the seven highest points in the peak. The seven-point group was selected so that the middle point had the highest value. The time at which the maximum was calculated from the quadratic fit was used as the retention time of the peak. Seven points were used, since this number represented a compromise between the optimum number of points for a peak with a τ/σ_G ratio of 1 (mildly skewed) and a peak with a τ/σ_G ratio of 4 (heavily skewed) for the data sampling rate used. This compromise was selected so that the quadratic fit could be used for peaks for which the value of τ/σ_G was not known, as in real chromatograms. The asymmetry of the peak was taken as the value of b/a, where a and b were determined as shown in Fig. 3 at the appropriate peak heights.

RESULTS AND DISCUSSION

Simulated peaks without noise

The values for the area and the variance obtained by the summation and width-asymmetry methods for simulated peaks and an ideal baseline are compared in Fig. 4 as a function of the percent valley between the peaks. For only slightly tailed peaks ($\tau/\sigma_G = 1$, Fig. 4a), it appears that the summation method for peak area and variance is fairly accurate for the noiseless peaks used in this study. However, for moderately tailed peaks ($\tau/\sigma_G = 2$, Fig. 4b), the area of the second peak and the variance of the first peak have become much less accurate relative to the same parameters measured by using the width-asymmetry method. This trend continues in Fig. 4c for $\tau/\sigma_G = 4$. Thus, the width-asymmetry method can be used to measure accurately both the area and variance for the left peak of a highly skewed and overlapped pair of peaks, while the same parameters cannot be measured as accurately for either peak when the summation method is used.

Fig. 5 illustrates the same results for peak area and variance as Fig. 4c, but with a less ideal baseline. Here, the variance for the first peak, as measured by the summation method, is very inaccurate. This is due to a significant portion of the tail of the first peak being truncated by the higher baseline, and shows the sensitivity of the variance to baseline errors when measured by the summation method. However, the variance measured by our width-asymmetry method does not show this sensitivity.

The errors in the higher moments, skew and excess are compared in Fig. 6 for an overlapped pair of highly skewed peaks ($\tau/\sigma_G = 4$) and an ideal baseline. Since an ideal baseline was simulated, the moments measured for the right peak by the summation method (see Fig. 6a) show a fair accuracy up to a high percent valley for these noiseless peaks. However, results this accurate cannot be expected for real chromatographic peaks, due to the problems outlined in the introduction for the summation method. As expected, the higher moments, skew and excess, measured by the summation method for the left peak, show greater sensitivity to the truncation of the peak tail than do the area and variance for the left peak, measured by the summation method.

However, the higher moments, skew and excess measured by the width-asymmetry method for the left peak do not show this sensitivity (see Fig. 6b). In fact, the accuracy for these parameters, measured by the width-asymmetry method, is much better than that obtained by the summation method for the right peak. This shows that the width-asymmetry method is better overall for measuring the higher statistical moments, excess and skew for at least one peak of an overlapped pair of peaks.

Table I shows the maximum percent valleys (maximum overlap) for which the two methods described here are in error by less than 5%. As seen there, for most of the moments calculated by the width-asymmetry method for the left peak, the maximum overlap that can be tolerated is higher. However, for the right peak, some of the moments calculated by the summation method are more accurate. These results were expected, since the tail for the right peak is fully included in the limits of integration. Peak overlap of the peaks prevents the tail of the left peak from being included in the summation method. This confirms the well-known results that the tail of a skewed peak is especially important in calculating the higher moments by the summation method. Also, the results show that the degree of distortion in the left peak is low for two overlapped EMG peaks. Overall, these results indicate that all the statistical

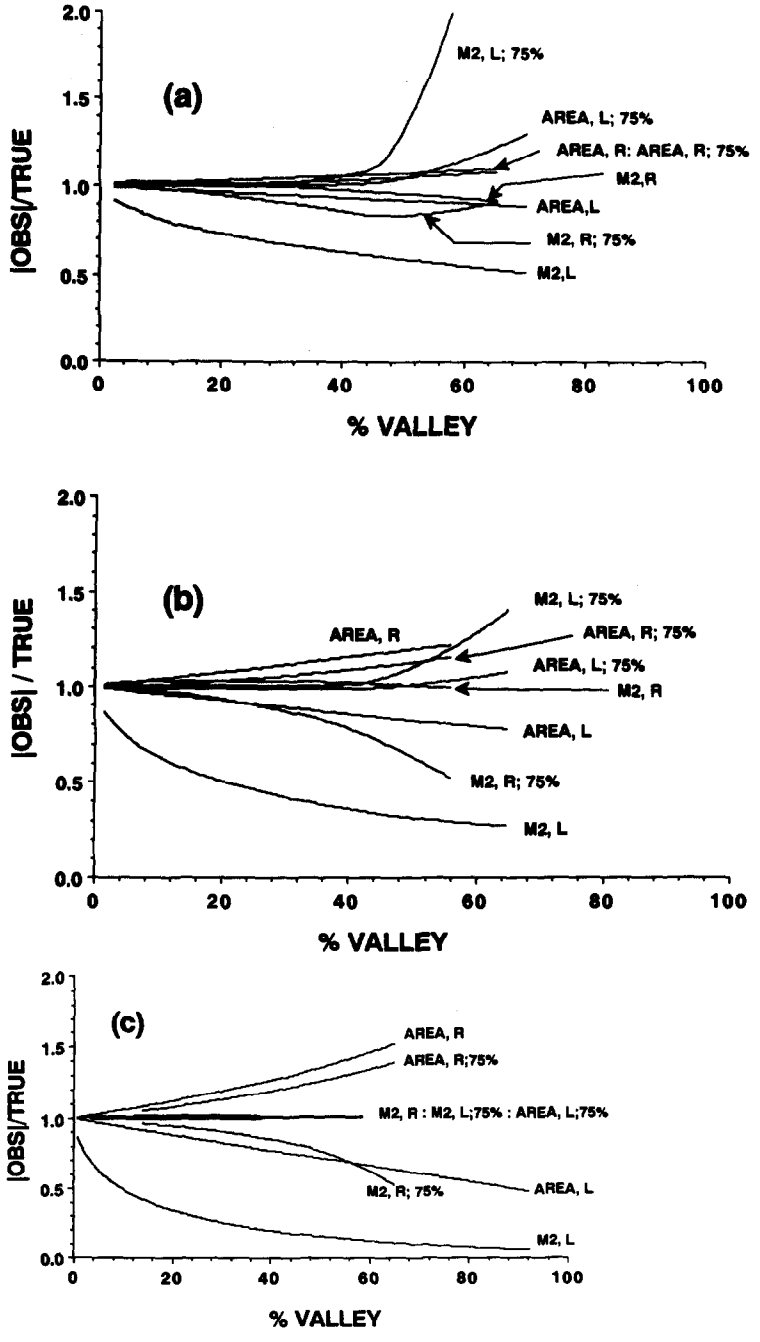

Fig. 4. Comparison of the errors in peak area and variance occurring in the summation and width-asymmetry methods as a function of peak overlap (percent valley) for: (a) $\tau/\sigma_G = 1$; (b) $\tau/\sigma_G = 2$ and (c) $\tau/\sigma_G = 4$. Labels in the plot refer to: (a) the parameter; (b) the peak (first, L, or second, R) for which a parameter was obtained and (c) the relative peak height at which the width and asymmetry were measured (width-asymmetry only). For example, "M2, L; 75%" refers to the variance measured for the first peak of the overlapped pair at 75% of the peak height, while "M2, R" refers to the variance of the second peak measured by the summation method.

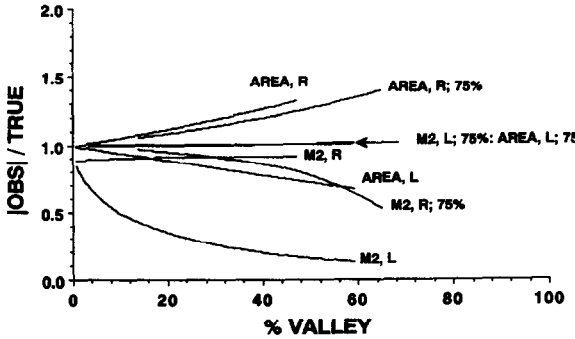

Fig. 5. Comparison of the errors in peak area and variance occurring in the summation and width-asymmetry methods as a function of peak overlap for a highly skewed pair of peaks ($\tau/\sigma_G = 4$) with a less than ideal baseline level (0.1%). Conditions as in Fig. 4.

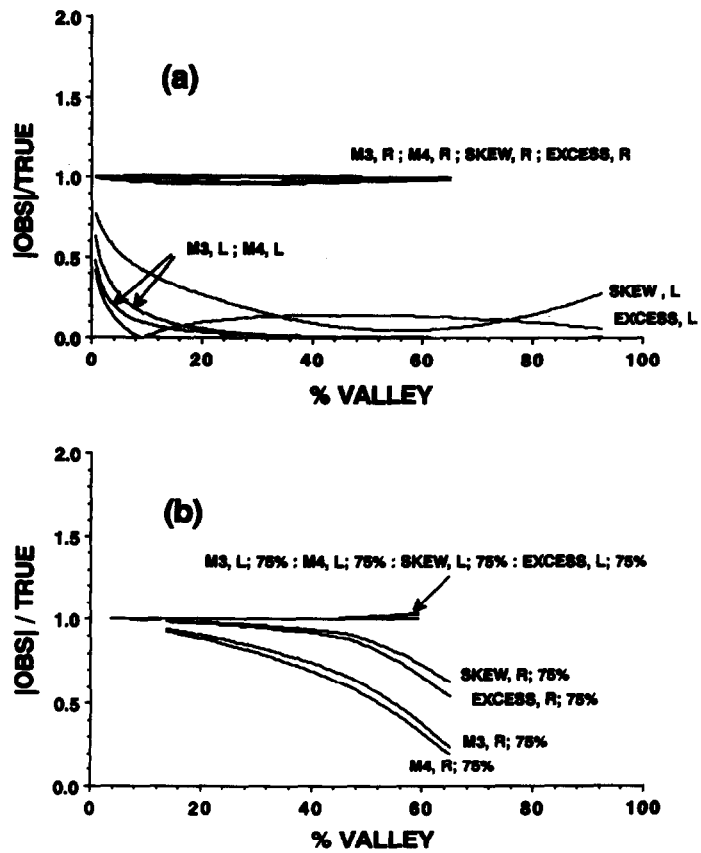

Fig. 6. Comparison of the errors in peak parameters other than peak area and variance for overlapped peak pairs with $\tau/\sigma_G = 4$ occurring in: (a) summation method and (b) width-asymmetry method. Conditions as in Fig. 4.

TABLE I

MAXIMUM PEAK OVERLAP (PERCENT VALLEYS) THAT CAN BE TOLERATED BY THE WIDTH-ASYMMETRY AND SUMMATION METHODS FOR A GIVEN ACCURACY (≤ 5% ERROR)

	Peak	*Area*	M_2	M_3	M_4	*Skew*	*Excess*
Width-asymmetry	First	47	34	16	24	17	15
method	Second	4	17	8	8	8	7
Summation method/	First	9	<2	<2	<2	<2	<2
ideal baseline*	Second	8	34	5	11	5	2
Summation method/	First	8	<2	<2	<2	<2	<2
non-ideal baseline**	Second	9	24	9	<2	9	<2

* Peak start/stop corresponds to points where signal is $5 \cdot 10^{-8}$% of maximum.
** Peak start/stop correspond to points where signal is 0.1% of maximum.

moments, including excess and skew can be measured accurately for peaks that are moderately overlapped, the more skewed peaks giving the best results. This latter trend is due primarily to the percent valley parameter, which tends to underestimate peak overlap for symmetrical peaks and to overestimate peak overlap for skewed peaks. (However, this measure of peak overlap is no worse than any other parameter, and is more practical than most other measures of peak overlap for skewed peaks[4].) Of course, Figs. 4–6 also show that for those overlapped peaks which are not highly skewed the summation method may occasionally give better results.

Although the summation method appears to be fairly accurate for the higher moments of the second peak of a moderately to highly skewed overlapping peak pair, it will generally be very inaccurate with real chromatographic data (peaks with noise). In modern chromatographic integrators and data systems, much of the tail of even a mildly skewed peak is often not included in the summation, due to baseline errors occurring when the algorithm used detects a peak stop before the actual end of the peak is reached. Many data systems rely on the first derivative, second derivative or similar tests to detect peak end with a slope sensitivity setting which depends on the degree of noise in the chromatogram[10]. The slope sensitivity is set at a level higher than what might be expected for the baseline drift. However, this setting may frequently also be higher than the slope on the tail of a skewed chromatographic peak. On overlapped pairs of peaks, the premature peak end would affect the second peak almost exclusively, therefore disallowing the use of the second peak in accurate computation by the summation method of the higher statistical moments for that peak, and often of the area and the second moment as well. In contrast, the width-asymmetry method is relatively unaffected by this type of truncation error[11]. Furthermore, in this example, the width-asymmetry method, which is applied to the first peak of an overlapped pair, would be entirely unaffected by the premature peak stop on the trailing edge of the second peak.

Results for real chromatographic peaks

Table II shows the results for an isolated, real chromatographic peak, obtained under ideal conditions [high signal-to-noise (S/N) ratios, no overlap, baseline

resolution, etc.]. As seen in Table II, the zeroth through fourth statistical moments, along with peak excess and skew for the single peak, were found to be similar for the width-asymmetry and summation methods. Under less ideal conditions, with a much smaller S/N ratio, the summation method would probably give results very different from the width-asymmetry method, due to the limitations of the summation method mentioned in the Introduction.

The appropriateness of the EMG model for this real chromatographic peak is demonstrated by the agreement obtained for the various peak parameters at different relative peak heights. Although the agreement is not exact, the spread in peak parameters is small (usually $< 5\%$) compared to the error encountered when using the summation method on most real peaks, the latter due to the problems outlined in the Introduction.

The advantage of the width-asymmetry method over the summation method for real peaks becomes apparent when overlapping peaks are examined. Table III gives the results for the summation and width-asymmetry methods for two pairs of peaks that overlap by different amounts. As seen for the 40% valley case, the summation method gave relative areas of 39.5% for the left peak and 60.5% for the right peak. Since the true relative areas of the peaks are 50%, the error in peak area for each peak is 10.5% when using the summation method and perpendicular drop algorithm. In contrast, the width-asymmetry method gave relative areas of 50.0% for each peak, *i.e.*, exactly the correct result.

For the more heavily overlapped peak pair (67% valley), the errors associated with the summation method increased, whereas the width-asymmetry method again gave results very close to the correct result (areas measured for the single peak shown in Table II). The relative areas for the left and right peaks, determined by the summation method, were in error by 17% for each peak, whereas the relative areas measured by the width-asymmetry method were in error by only 1%. The other statistical moments, including skew and excess for the left peak, show a large difference between those calculated by the width-asymmetry and summation methods. Also, a comparison of these parameters measured for the left peak by using the width-asymmetry method to those for the single, isolated peak (see Table II) shows that the width-asymmetry method gave very good results.

TABLE II

COMPARISON OF SUMMATION AND WIDTH-ASYMMETRY METHODS FOR AN ISOLATED REAL CHROMATOGRAPHIC PEAK

	Height (%)	*Peak area (mV · s)*	M_1	M_2	M_3	M_4	*Skew*	*Excess*
Summation method	Not applicable	5449.6	1.718	0.0424	0.0186	0.0216	2.146	8.974
Width-asymmetry method	10	5370.0	1.722	0.0410	0.0149	0.0138	1.794	5.191
	25	5530.7	–	0.0440	0.0167	0.0159	1.807	5.242
	50	5870.8	1.892	0.0468	0.0184	0.0181	1.817	5.278
	75	5802.7	–	0.0500	0.0205	0.0208	1.828	5.321

TABLE III

COMPARISON OF SUMMATION AND WIDTH-ASYMMETRY METHODS FOR TWO SETS OF OVERLAPPING, REAL PEAKS

Overlapping peaks generated from rapid, duplicate injections of pyrene (see Experimental section). Therefore, values for all the parameters except M_1 should be the same for all peaks within experimental error.

	Height (%)	*Peak area (mV · s)*	M_1	M_2	M_3	M_4	*Skew*	*Excess*
Summation method								
*40% Valley**								
Left peak	Not applicable	4481.4	1.633	0.0093	$3.7 \cdot 10^{-5}$	$1.9 \cdot 10^{-4}$	−0.795	0.042
Right peak	Not applicable	6866.8	2.101	0.0501	0.0319	0.0469	2.839	15.669
*67% Valley**								
Left peak	Not applicable	3733.6	1.609	0.0061	$-6.13 \cdot 10^{-5}$	$5.1 \cdot 10^{-5}$	−1.626	−0.130
Right peak	Not applicable	7347.5	1.997	0.0416	0.0175	0.0179	2.067	7.340
Width-asymmetry method								
*40% Valley**								
Left peak	50	5672.7	–	0.0405	0.0140	0.0130	1.720	4.907
	75	5676.8	–	0.0419	0.0150	0.0141	1.744	5.000
Right peak**	50	5675.5	–	0.0371	0.0118	0.0105	1.654	4.656
	75	5671.4	–	0.0354	0.0113	0.0098	1.690	4.794
*67% Valley**								
Left peak	50	–	–	–	–	–	–	–
	75	5694.2	–	0.0454	0.0170	0.0166	1.761	5.063
Right peak**	50	–	–	0.0218	0.0024	0.0020	0.746	1.612
	75	5386.9	–	0.0258	0.0062	0.0047	1.483	4.025

* Valley height relative to left (first) peak.

** Areas calculated as $A_R = (A_L + A_R)_{summation} - A_L$; other parameters calculated using the width-asymmetry equations in Fig. 1b. Width-asymmetry equations should normally not be employed for the more distorted peak of an overlapping pair of peaks. Results are shown here for purposes of comparison only.

Modified width-asymmetry method for true peak deconvolution

In order to determine relative areas, the width-asymmetry method requires knowledge of the total area of the two overlapping peaks, as measured by the summation method. The total area cannot be calculated by the width-asymmetry method, because the distortion of the right peak by the tail of the left peak causes an erroneous contribution to the total area. Thus, the width-asymmetry method, as employed until now, is not a true peak deconvolution method, since parameters other than the peak area are not additive and therefore cannot be determined for the second peak by subtraction. However, the width-asymmetry method can be modified and used to deconvolve an overlapping pair of peaks as follows: first, the first peak of the overlapping pair is calculated point by point via eqn. 1 over an appropriate time interval from values of σ_G, τ and A that are estimated from peak width, asymmetry and peak-height (area only) measurements by using equations described elsewhere[5]. Next, t_G is estimated from $M_1 - \tau$ and then adjusted so that the maxima of the calculated peak coincides exactly with the maxima of the first peak in the overlapping pair of real peaks. Finally, the second peak of the overlapped pair is obtained by subtracting, point by point, the calculated values of the first peak from the total chromatographic signal.

The accuracy of the width-asymmetry deconvolution method is evident from Table IV for real chromatographic peaks. As Tables III and IV show, both the original width-asymmetry method and the modified width-asymmetry/deconvolution method give accurate results for all parameters for the first peak of the overlapped pair. Note, however, that the original width-asymmetry method uses values of peak height, width and asymmetry obtained directly from the actual chromatogram (at 75% relative peak height), whereas the width-asymmetry/deconvolution method uses the same values from an artificially constructed peak.

Fig. 7 illustrates the results of the width-asymmetry deconvolution method, applied to the chromatogram of overlapped peaks with the 40% valley (relative to the left peak); superimposed on the real chromatogram are the two simulated peaks, obtained from the width-asymmetry deconvolution method. As seen, the deconvoluted first peak falls directly on the actual first peak of the overlapped pair. This was expected, since there is little distortion of the first peak in the overlapped pair from the second peak. The distortion of the second peak in the overlapped pair, caused by the tail of the first peak, is readily apparent, though, as the difference in both height and area of the second peak and its corresponding deconvoluted peak is large. This distortion is the reason that the perpendicular drop method underestimates the area of the first peak and overestimates the area of the second peak in an overlapped pair of peaks. It is also why, as mentioned above and shown here, the width-asymmetry method cannot be applied directly to the second peak of an overlapped pair of peaks.

Computational time

As stated in the Introduction, one problem with the summation method is that every point in the chromatographic peak must be involved in moment calculations. However, when the width-asymmetry method is used, most of the points in a peak of interest do not have any calculations performed on them. When the two methods were timed against each other, the width-asymmetry method was found to be about twice as fast for single-peak chromatograms and up to ten times faster for multiple-peak chromatograms.

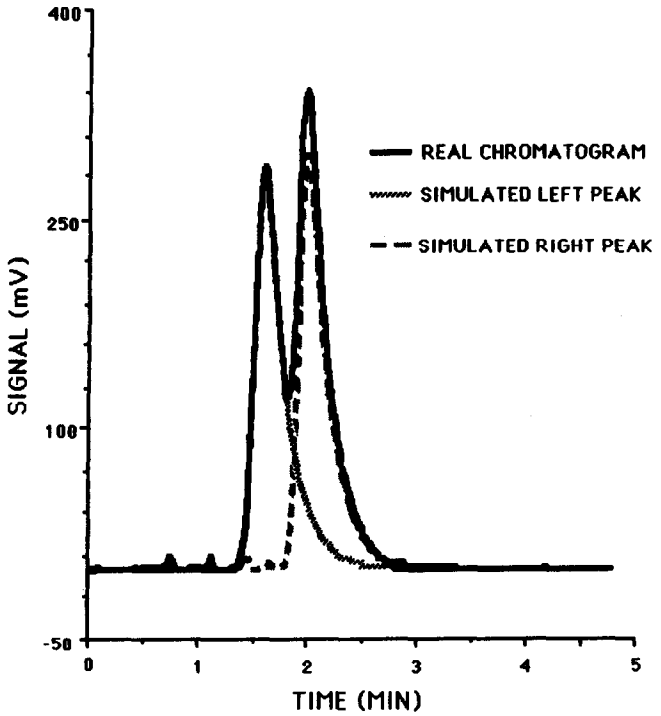

Fig. 7. Visual interpretation of the width-asymmetry/deconvolution method. The solid black line indicates the real overlapping chromatographic peaks, while the lighter lines show the individual peaks that are predicted by the width-asymmetry/deconvolution method.

TABLE IV

RESULTS FOR MODIFIED WIDTH-ASYMMETRY/DECONVOLUTION METHOD FOR TWO SETS OF OVERLAPPING, REAL PEAKS

Conditions as in Table III. Values for each parameter were calculated from width-asymmetry equations at 10, 25, 50 and 75% relative peak height. The values obtained were then averaged for this table. The spread of values for any parameter never exceeded 5% for the left peak and 10% for the right peak.

	Peak area *(mV · s)*	M_1	M_2	M_3	M_4	*Skew*	*Excess*
40% Valley							
Left peak	5654.4	1.798	0.0415	0.0147	0.0138	1.738	4.976
Right peak	5598.1	2.197	0.0411	0.0150	0.0139	1.804	5.229
67% Valley							
Left peak	5676.4	1.827	0.0450	0.0168	0.0164	1.755	5.042
Right peak	5272.2	2.090	0.0356	0.0120	0.0104	1.780	5.138

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of this work by the Computer-Aided Chemistry Department of the Perkin-Elmer Corporation and the National Oceanographic and Atmospheric Administration (NOAA). Mark S. Jeansonne also thanks Jeffrey Crow for his helpful discussion on this topic.

REFERENCES

1 S. N. Chesler and S. P. Cram, *Anal. Chem.*, 43 (1971) 1922–1933.
2 T. Petitclerc and G. Guiochon, *J. Chromatogr. Sci.*, 14 (1976) 531–535.
3 E. Grushka, M. N. Myers and P. D. Schettler, *Anal. Chem.*, 41 (1969) 889–892.
4 J. P. Foley, *J. Chromatogr.*, 384 (1987) 301–313.
5 J. P. Foley and J. G. Dorsey, *J. Chromatogr. Sci.*, 22 (1984) 40–46.
6 R. E. Pauls and L. B. Rogers, *Anal. Chem.*, 49 (1977) 625–628.
7 E. Grushka, *Anal. Chem.*, 44 (1972) 1733–1738.
8 J. P. Foley, *Anal. Chem.*, 59 (1987) 1984–1987.
9 J. P. Foley and M. S. Jeansonne, manuscript in preparation.
10 A. L. Colmsjo, *Chromatographia*, 23 (1987) 257–260.
11 D. P. Anderson and R. R. Walters, *J. Chromatogr. Sci.*, 22 (1984) 353–359.